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TITLE: The Effect of Hypobaria on Muscle Inflammation and Regeneration After Injury and Hemorrhagic Shock

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14. ABSTRACT

The purpose of this research is to understand the effect of long-distance flying on recovery after muscle injury and significant bleeding. This situation may affect recovery following combat injuries, especially if wounded service members are traveling from Asia to the United States. To date, we have tested the hypothesis that there will be no difference in well-being and white blood cells populations in skeletal muscle between male mice exposed to hypobaria for 16 hours and male mice exposed to normobaria. This hypothesis was supported. This finding suggests that long distance flying alone does not induce obvious physiological effects or affect the presence of white blood cells normally present in skeletal muscle.

15. SUBJECT TERMS
Aeromedical evacuation
En route care
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1.0 INTRODUCTION

En route care is a critical and successful strategy for the early intervention of life-threatening battle injuries. Wounded warfighters typically receive en route care during the flight from the battlefield to the nearest medical facility, which averages 8 hours in current military operations. The issue with this process is the cabin of a medical transport plane is pressurized to 8,000 feet, meaning wounded service members in transit and without supplemental oxygen are exposed to less oxygen than if transported by ground. Furthermore, future combat operations are expected to emerge from Asia, which would double the transit time from the battlefield to a medical care facility. While injury recovery in low oxygen environments requires further analysis, military operations are not slowing but expanding to more remote areas. Therefore, this study will evaluate the physiological effects of air transport in low oxygen environments.

This project aims to examine patient recovery after prolonged exposure to low oxygen levels; identify the effects of exposure; and determine appropriate countermeasures and treatments, if necessary. Laboratory mice will be used to determine exposure effects because their genetic and biological processes mirror those of humans. This approach will allow an adequate assessment of recovery without harming human patients.

Three hypotheses guide this project.

(a) Mice exposed to atmospheric pressure equivalent to that of an airplane cabin—i.e., hypobaria—will have muscle-based (intramuscular) white blood cell numbers and activity similar to mice exposed to normal atmospheric pressure.
(b) Mouse muscle recovery and systemic inflammatory status will vary, depending on the type of resuscitation fluid used after undergoing crush muscle injury and hemorrhagic shock.
(c) Hypobaria starting at 24 hours after crush muscle injury and hemorrhagic shock with fluid resuscitation will lead to slower muscle recovery.

Overall, this research may lead to improvements in wounded service members' care during air transport and their recuperation after treatment.

2.0 KEYWORDS

Aeromedical evacuation
En route care
Hemorrhagic shock
Hypobaria
Inflammation
Skeletal muscle injury
Skeletal muscle regeneration

3.0 ACCOMPLISHMENTS

3.1 Major Year 01 Project Goals

The status of the major Year 01 project goals is summarized in Table 1. The accomplishment details are provided in section 3.2.

Table 1. Major Year 01 Project Goals

	Month Completed	% Completed
Major Task 1: Local IACUC Approval		
Subtask 1: Develop University of Nevada, Las Vegas (UNLV), Institutional Animal Care and Use (IACUC) animal protocol.	October 2016	100%
Subtask 2: Provide information about hemorrhagic shock and fluid resuscitation model.	October 2016	100%
Subtask 3: Provide statistics-related information.	Not applicable	Not applicable
Milestone Achieved: Local IACUC approval received.	November 2016	
Major Task 2: Animal Care and Use Review Office (ACURO) Approval		
Subtask 1: Develop ACURO protocol.	November 2016	100%
Subtask 2: Provide information about hemorrhagic shock and fluid resuscitation model.	October 2016	100%
Subtask 3: Provide statistics-related information.	Not applicable	Not applicable
Milestone Achieved: ACURO approval received.	December 2016	100%
Major Task 3: Personnel Hiring and Training		
Subtask 1: Hire personnel.		95%
Subtask 2: Orient/train personnel as needed.		80%
Milestones Achieved: Personnel oriented and trained.		85%
Major Task 4: Hypotheses 1 and 2 Testing		
Subtask 1: Complete Hypothesis 1 animal procedure (22 C57BL/6 male mice).	February 2017	100%
Subtask 2: Perform Hypothesis 1 analysis.		90%
Subtask 3: Set up hemorrhagic shock and fluid resuscitation model (10 C57BL/6 male mice).		50%
Subtask 4: Perform Hypothesis 2 animal procedures (100 C57BL/6 male mice).		0%
Subtask 5: Perform Hypothesis 2 analysis.		0%
Subtask 6: Review Hypothesis 1 and 2 results.		40%
Subtask 7: Write and/or review report.		40%
Subtask 8: Disseminate results.		0%
Subtask 9: Submit and receive animal protocol amendment approval.		85%
Milestone Achieved: Hypothesis 1 and 2 testing and animal amendment approval completed.		50%

3.2 Accomplishment Details

3.2.1 Major Task 1: Local IACUC Approval – Completed on 15-November-2016.

3.2.2 Major Task 2: ACURO Approval – Completed on 21-December-2016.

3.2.3 Major Task 3: Personnel Hiring and Training

Subtask 1: Hire personnel.
Table 2 lists the personnel hired in Year 01.

Table 2. Personnel Hired in Year 01

Name	Start Date	Role
Dr. Barbara St. Pierre Schneider	20-July-2016	Principal Investigator
Daniela Rincon Cornejo	01-December-2016	Project Coordinator
Dr. Liyuan (Angi) Zhang[a]	01-January-2017	Postdoctoral Scholar
Dr. Jessica Muniga[b]	02-February-2017	Research Veterinarian
Dr. Zhuowei Li	03-March-2017	Research Technician
Theofania Mavrantonis	22-May-2017	Student Worker
Hanine Derkhshan	25-May-2017	Student Worker
Dr. Kelley Hammond	07-August-2017[c]	Postdoctoral Scholar

[a]Dr. Zhang received her H-1B visa on 03-May-2017.
[b]Since her start date, Dr. McMorris changed her surname to Muniga.
[c]Dr. Hammond was hired on 26-June-2017, but her start date is delayed because of the completion of her PhD.

During Year 01, quarter 4, we also advertised and conducted two phone interviews for the two graduate assistant (GA) positions. On 21-July-2017 and 24-July-2017, two GA applicants were interviewed at UNLV. The applicants accepted the positions on 24-July-2017 and 25-July-2017. The start date for these two GAs is 22-August-2017.

Subtask 2: Orient/train personnel as needed.
We oriented and trained one project coordinator, one postdoctoral scholar, one research veterinarian, one animal research technician, and two student workers.

3.2.4 Major Task 4: Hypotheses 1 and 2 Testing

Subtask 1: Complete Hypothesis 1 animal procedure – Accomplished on 28-February-2017.

Subtask 2: Perform Hypothesis 1 analysis.
By the end of Year 01, quarter 4, the following tasks were accomplished for this subtask:

(a) analyzed the general morphology of the muscle of mice exposed to normobaria and hypobaria;
(b) completed data collection for the study variables, CD206-positive leukocytes and CD68-positive leukocytes. (In the original proposal, we planned to collect data related to CD68-positive leukocytes. However, because these cells were not abundant in the muscle, we

collected data related to CD206-positive cells. For more details about this issue, please see section 5.2.); and

(c) compiled and emailed the following Hypothesis 1 data to the statistician: chamber CO_2 levels; chamber pressure levels; mouse body weight before and after exposure to normobaria and hypobaria; and CD206 number, mean CD206 antigen area, and CD206 area percentage.

Subtask 3: Set up hemorrhagic shock and fluid resuscitation model.
As part of this task, the research veterinarian completed hemorrhagic shock and fluid resuscitation model training at the University of Cincinnati. This training was required by the UNLV IACUC because the veterinarian had not performed these procedures before. Through this training, we have established competency in catheterizing the femoral artery with polyethylene (PE) tubing for withdrawing blood and instilling fluids.

After the University of Cincinnati training, we determined that changes in technique and additional femoral artery catheterization practice and training were needed. With approval from the UNLV IACUC and ACURO, 40 additional development (nonsurvival) mice were added for practice and training purposes. The testing of these mice was completed on 30-June-2017 and resulted in a 95% success rate in catheterization of the femoral artery using PE tubing.

Subtask 4: Perform Hypothesis 2 animal procedures.
We did not start this task because of the delay in personnel hiring, the delay in starting Hypothesis 1, and the training needed to set up the hemorrhagic shock and fluid resuscitation model. We estimate starting this task as soon as the Las Vegas temperature falls below 85 degrees Fahrenheit. According to the animal vendor, mice can be shipped to Las Vegas only at this temperature range. The possibility of this heat embargo was explained in the grant proposal.

Subtask 5: Perform Hypothesis 2 analysis.
This subtask has been delayed because of the delay in subtask 4.

Subtask 6: Review Hypothesis 1 and 2 results.
We started reviewing the Hypothesis 1 results as soon as we completed testing on each mouse. To date, the Hypothesis 1 results are as follows:

(a) no difference in the well-being of mice exposed to normobaria at 14 to 17 hours of hypobaria,
(b) no difference in general muscle morphology between normobaric and hypobaric mice,
(c) no difference in the presence of intramuscular CD206-positive cells between normobaric and hypobaric mice, and
(d) no difference in the presence of intramuscular CD68-positive cells between normobaric and hypobaric mice.

Therefore, Hypothesis 1 was supported.

Since we were delayed in starting Hypothesis 2, this part of subtask 6 is delayed.

Subtask 7: Write and/or review report.
This report will consist of two sections: Hypothesis 1 and Hypothesis 2. We have written the first draft of the Hypothesis 1 section, and we have completed the following subsections: Introduction, Methods, Figures, and References. We have experienced a delay in completing a final Hypothesis 1 section report because of three major reasons:

(a) room temperature issues led to repeating most of the CD206 and CD68 immunolabeling, which delayed starting the report;
(b) unanticipated training related to producing high quality images, organizing data, and ensuring rigor was needed; and
(c) we require the statistical analysis to complete the report, which—as of 27-July-2017—is 40% complete.

We estimate completing this section of the report by 15-September-2017.

Subtask 8: Disseminate results.
Nothing to report

Subtask 9: Submit and receive animal protocol amendment approval.
See section 5.4.

3.3 Training and Professional Development Opportunities
All personnel were exposed to opportunities for training as part of professional development.

Dr. Barbara St. Pierre Schneider, Principal Investigator
Activities completed: IACUC refresher training

Dr. Jessica Muniga, Research Veterinarian
Activities completed: IACUC training, mouse training (including harvesting tissue and blood collection), PE catheter insertion, hypobaria, Millar Mikro-Tip® catheter handling, Power Lab software training, and plantarflexor muscle function testing

Dr. Zhuowei Li, Research Technician
Activities completed: IACUC training, mouse training (including harvesting tissue and blood collection), Millar Mikro-Tip® catheter handling, Power Lab software training, and plantarflexor muscle function testing

Dr. Liyuan (Angi) Zhang, Postdoctoral Scholar
Activities completed: IACUC training, mouse training (including harvesting tissue and blood collection), immunohistochemistry, cryosectioning, data organization and rigor, and imaging analysis software

Daniela Rincon Cornejo, Project Coordinator
Activities completed: increased understanding in managing a large Department of Defense (DoD) grant, UNLV purchasing and accounting software, UNLV Human Resources procedures, UNLV Facilities procedures, team building activities, IACUC training, and mouse handling

3.4 Dissemination of Results
Nothing to report

3.5 Year 02 Plans

During Year 02, we plan to complete the major tasks of specific aim 1: to establish a combat casualty/en route care experimental model that incorporates lower extremity muscle injury and hemorrhagic shock with fluid resuscitation and simulates the hypobaria exposure of air transport from the Pacific theater to the United States.

Next, we plan to start the major tasks of specific aims 2 and 3.
Specific aim 2: To test the effect of 16 hours of hypobaria exposure on leukocyte characteristics, function, and related genes responsive to muscle injury in a combat casualty/en route care experimental model

Specific aim 3: To test the effect of 16 hours of hypobaria exposure on muscle regenerative processes and functional properties in a combat casualty/en route care experimental model

The details of this plan are described below.

Specific Aim 1, Major Task 3: Personnel Hiring and Training

Subtask 1: Hire personnel.
The second postdoctoral scholar will start on 07-August-2017, and the two GAs will start on 22-August-2017.

Subtask 2: Orient/train personnel as needed.
The second postdoctoral scholar and GAs will be trained by 27-October-2017.

Specific Aim 1, Major Task 4: Hypotheses 1 and 2 Testing

Subtask 2: Perform Hypothesis 1 analysis.
The following tasks will be accomplished:

(a) compile and email the remaining Hypothesis 1 data to the statistician: CD206 number per square millimeter, CD68 number, CD68 number per square millimeter, mean CD68 antigen area, and CD68 area percentage; and
(b) complete statistical analysis of the Hypothesis 1 data.

We estimate that this task will be completed by 15-September-2017.

Subtask 3: Set up hemorrhagic shock and fluid resuscitation model.
The following tasks are planned:

(a) Seek UNLV IACUC and ACURO approval to add 10 more development (nonsurvival) mice to develop competency in inserting the Millar Mikro-Tip® catheter into the carotid artery and 10 more refinement (survival) mice to ensure that mice recover well from both carotid and femoral artery catheterizations.

    We submitted this protocol amendment to UNLV IACUC on 11-July-2017 and received approval on 21-July-2017. We also submitted this amendment to ACURO on 03-August-2017 and received approval on 09-August-2017.

(b) Seek UNLV IACUC and ACURO approval for a standard operating procedure for expired drugs, stock solutions, and fluid bags.

We submitted this protocol amendment to UNLV IACUC on 27-July-2017 and received approval on 07-August-2017. We plan to submit this amendment to ACURO by 31-August-2017.

(c) Receive training at the University of California, Los Angeles (UCLA), Mouse Physiology Laboratory with expertise in Millar Mikro-Tip® catheter insertion on 08-August-2017.

This training was sought because, following practice and training with PE tubing, we were unsuccessful in inserting the Millar Mikro-Tip® catheter into the femoral artery of the mice. (The Millar Mikro-Tip® catheter will be used to monitor arterial pressure during hemorrhagic shock and fluid resuscitation.) Prior to this training, we believed the lack of success in inserting this catheter could be related to our lack of catheter insertion experience, the unsuitability of this catheter for the mouse femoral artery, and/or the flimsiness of the catheter. We contacted the vendor several times for troubleshooting and technical support, but this support was not adequate. Therefore, we arranged training with the UCLA Mouse Physiology Laboratory on 14-July-2017, which occurred on 08-August-2017.

The UCLA training was completed, and we were successful in inserting the Millar Mikro-Tip® catheter into the mouse femoral artery. During this training, we learned that heavier mice (~30 g body weight) and additional surgical instruments need to be used with this catheter. Therefore, we have ordered these instruments and will ensure that the mouse body weight is ~30 g prior to starting the hemorrhagic shock/fluid resuscitation procedures.

(d) Submit protocol amendments to UNLV IACUC and ACURO to add new personnel (one postdoctoral scholar and two GAs) and adjust parameters of the plantarflexor muscle function testing procedure.

We anticipate submitting this amendment by 31-August-2017.

(e) Submit the refinement mouse order upon receiving these approvals.

This mouse order was submitted to the vendor on 11-August-2017. However, we received notice that the mice could not be shipped because of the shipping heat embargo, which is described in section 3.2.4. We will continue to submit the mouse order as August is monsoon season in Nevada, and the possibility exists that a temporary temperature drop may occur. Once we receive the mice and their body weight reaches ~30 g, we will test these mice over a one-week period.

Subtask 4: Perform Hypothesis 2 animal procedures.
We estimate starting this task in September 2017, or as soon as the refinement mice are completed. We estimate completing this task by 30-November-2017.

Subtask 5: Perform Hypothesis 2 analysis.
We estimate starting this task in September 2017 and completing this task by 30-March-2018.

Subtask 6: Review Hypothesis 1 and 2 results.
For Hypothesis 1, we plan to review the statistical analysis results upon completion. We estimate completing this task for Hypothesis 1 by 15-September-2017.

For Hypothesis 2, we estimate starting this task in September 2017 and completing this task by 30-March-2018.

Subtask 7: Write and/or review report.
We estimate completing the Hypothesis 1 section by 15-September-2017. The remaining tasks are as follows:

(a) complete the Results and Discussion sections,
(b) modify References as needed, and
(c) write an abstract.

For Hypothesis 2, we estimate completing this task by 31-July-2018.

Subtask 8: Disseminate results.
We plan to submit the Hypothesis 1 results to the journal, *Nursing Research*, and we plan to submit an abstract to the 2018 Military Health System Research Symposium. If we have compelling preliminary Hypothesis 2 results, we plan to present these results at a trauma-related or physiology-related conference.

Subtask 9: Submit and receive animal protocol amendment approval.
For Year 02, quarter 1, we anticipate additional UNLV and ACURO amendments. UNLV IACUC amendment number 5 (package number 834441-9) was approved on 21-July-2017 and represents changes in hemorrhagic shock and fluid resuscitation and plantarflexor muscle function testing procedures, the use of development/refinement mice, and personnel. This amendment was submitted to ACURO on 03-August-2017 and approved on 09-August-2017.

Amendment number 6 (package number 834441-10) was submitted to and approved by UNLV IACUC to incorporate a standard operating procedure for expired drugs, stock solutions, and fluid bags. We plan to submit this amendment to ACURO by 31-August-2017.

Additional protocol amendments to UNLV IACUC and ACURO will involve adding new personnel (one postdoctoral scholar and two GAs) and adjusting parameters of the plantarflexor muscle function testing procedure. We anticipate submitting this amendment by 31-August-2017.

Major Task 1: Hypothesis 3 Testing
Subtask 1: Complete animal procedures.
We estimate that this task will start in December 2017 and continue through the rest of Year 02.

Subtask 2: Complete flow cytometry, immunohistochemistry, enzyme-linked immunosorbent (i.e., ELISA), multiplex, and reverse transcription polymerase chain reaction (RT-PCR) assays (including data entry) for mice.

For this task, the postdoctoral scholars will require flow cytometry training, which is planned for October 2017. We estimate that this task will start in December 2017 and continue through the rest of Year 02.

4.0 IMPACT

4.1 Impact on the Development of the Principal Discipline(s) of the Project

During this year, we have expanded knowledge regarding the presence of macrophages, a specific type of white blood cell, within the hindlimb muscle of healthy mice. We have determined that 14 to 17 hours of exposure to atmospheric pressure equivalent to that present in an airplane used to transport wounded soldiers does not affect the presence of macrophages within hindlimb muscle of healthy mice.

4.2 Impact on Other Disciplines

Nothing to report

4.3 Impact on Technology Transfer

Nothing to report

4.4 Impact on Society Beyond Science and Technology

Nothing to report

5.0 CHANGES/PROBLEMS

5.1 Changes in Approach and Reasons for Change

5.1.1 Year 01, Quarters 1 through 3

The following changes in approach and reasons were reported in the quarterly reports:

(a) housing of the mice,
(b) Hypothesis 1 leukocyte detection (i.e., inclusion of CD206),
(c) addition of development mice for preliminary femoral artery catheterization work for Hypothesis 2, and
(d) the technique of femoral artery catheterization for Hypothesis 2.

5.1.2 Year 01, Quarter 4

The following changes in approach have been made (Table 3). These changes are minor in that our hypotheses and objectives remain the same.

Table 3. Year 01, Quarter 4, Changes in Approach

Change	Rationale	Notes
Hypothesis 1: Use a customized image analysis program to quantify leukocytes instead of the commercial Image-Pro Premier 9.2 software.	This customized image analysis program generates results faster and is tailored to the unique features of the samples.	Dr. Shelley Jorgensen was updated regarding this change on 17-July-2017.
Use the carotid artery as the insertion site for the Millar Mikro-Tip® catheter.	The carotid artery, which has a larger lumen than the femoral artery, may be more suitable for the Millar Mikro-Tip® catheter.	Dr. Shelley Jorgensen was updated regarding this change on 17-July-2017.

5.2 Actual or Anticipated Problems or Delays and Actions or Plans to Resolve Them
As indicated in the quarterly reports, our major problems or delays have pertained to personnel hires and experimental approach. These problems/delays have affected our proposed timeline.

Regarding personnel, the corrective actions outlined in the quarterly reports resulted in the hiring of four of the five full-time personnel by 03-March-2017. During the fourth quarter, we filled the second and last postdoctoral position, and this individual will start on 07-August-2017. In addition, we began the search to fill the two GA positions. Based on preliminary GA applicant interviews, we anticipate hiring two GAs at the start of the fall academic semester, which is 21-August-2017. By the end of August, we expect to have all significant personnel hired.

Regarding experimental approach, we reported four problems. First, we experienced a delay in receiving initial animal protocol approval from the UNLV IACUC. However, with revisions, we were successful in receiving this approval on 15-November-2016.

A second problem involved prolonged Hypothesis 1 analysis. We proposed to examine the presence of CD68-positive leukocytes in the muscle of mice exposed to normobaria and hypobaria, but we discovered that the cells were sparser than we had anticipated. Therefore, we switched to detecting CD206-positive cells. While detecting CD206-positive cells, we learned the room temperature of the laboratory was excessively low. After UNLV Facilities adjusted the cooling system so the room temperature was between 72 and 75 degrees Fahrenheit, we repeated the detection of both CD206-positive and CD68-positive cells to ensure rigor and reproducibility. These events combined delayed our Hypothesis 1 analysis.

The delay in Hypothesis 1 analysis caused a third problem: a delay in completing the Hypothesis 1 report. To complete the Hypothesis 1 report, we need the statistical analysis. While we have emailed the statistician the Hypothesis 1 data, these data were not received in sufficient time for the statistician to complete all the analysis by 19-July-2017. As of 27-July-2017, the statistician has completed 40% of the statistical analysis. We estimate the statistician will complete the analysis by 31-August-2017, which will allow us to complete our report by 15-September-2017.

We encountered a fourth problem in setting up the hemorrhagic shock/fluid resuscitation models. Initially, the UNLV IACUC required that the research veterinarian receive femoral artery catheter insertion training. While this training was completed on 22-March-2017, the research veterinarian required additional practice, and changes in technique were needed. Therefore, we sought UNLV IACUC and ACURO approval for 40 development (nonsurvival) mice and changes in technique. Upon receipt of these approvals and during the testing of these development mice, the research veterinarian sought additional catheter insertion training. Collectively, these events led to a delay in completing the testing of the 40 development mice. This testing was completed on 30-June-2017 and resulted in a 95% success rate in catheterization of the femoral artery using PE tubing.

In contrast, consistent catheterization of the femoral artery using the Millar Mikro-Tip® catheter has not been as successful. We consulted the vendor, who provided limited troubleshooting and technical support. We also consulted the literature and other investigators who use the catheter for a similar purpose as ours, and we learned that this catheter may not be suitable for the mouse femoral artery. As explained in section 3.5, we have addressed this problem in multiple ways. Our corrective actions to successfully insert the Millar Mikro-Tip® catheter are to catheterize the femoral artery of mice with a body weight of ~30 g and use surgical instruments recommended by the UCLA Mouse Physiology Laboratory.

As indicated, these problems/delays have affected our proposed timeline. Strategies to reduce and/or avoid additional timeline delays are as follows:

(a) arrange for cross-training to ensure that multiple team members can perform the mouse and tissue analysis procedures,
(b) duplicate anesthesia set-up to have the capacity to anesthetize multiple mice simultaneously, and
(c) ensure that procedures are efficient.

5.3 Changes That Had a Significant Impact on Expenditures

The delay in hiring personnel has significantly impacted expenditures in that Year 01 expenditures are behind. The estimate is that the Year 01 expenditures are 6 months delayed.

5.4 Significant Changes in Use or Care of Vertebrate Animals

This project involves the use and care of mice. The initial animal protocol was approved by the UNLV IACUC on 15-November-2016 and designated by UNLV IACUC as package number 834441-3. This protocol was approved by ACURO on 21-December-2016. Table 4 lists the amendments by the UNLV IACUC-designated package number and provides a succinct description of each amendment.

Table 4. Year 01 Animal Protocol Amendments and Approval Dates

UNLV IACUC-Designated Package Number	UNLV Amendment Number	UNLV IACUC Approval Date	ACURO Amendment Number	ACURO Approval Date	Brief Amendment Description
834441-4	1	01-Feb-2017	1	10-Feb-2017	Clarification of Hypothesis 1 procedure and personnel
834441-5	2	18-April-2017	2	20-Apr-2017	Revision of hemorrhagic shock and fluid resuscitation procedures, development/refinement mice, and personnel
834441-7[a]	3	12-May-2017	3	22-May-2017	Change in drugs and hemorrhagic shock and fluid resuscitation and muscle function testing procedures
834441-8	4	05-June-2017	4	20-June-2017	Personnel

[a]834441-6 was withdrawn before UNLV IACUC review.

6.0 PRODUCTS

6.1 Publications, Conference Papers, and Presentations
Nothing to report

6.2 Journal Publications
Nothing to report

6.3 Books or Other Non-periodical, One-time Publications
Nothing to report

6.4 Other Publications, Conference Papers, and Presentations
Nothing to report

6.5 Website(s) or Other Internet Site(s)
Nothing to report

6.6 Technologies or Techniques
Nothing to report

6.7 Inventions, Patent Applications, and/or Licenses
Nothing to report

6.8 Other Products
Nothing to report

7.0 PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

7.1 Project Members
Table 5 lists the individuals who have worked on this project.

Table 5. Project Team Members

Name	**Dr. Barbara St. Pierre Schneider**
Project Role	Principal Investigator
Researcher Identifier	Not applicable
Nearest person month worked	9
Contribution to Project	Dr. Schneider assisted with personnel training and analysis, and she oversaw the project by ensuring that all personnel completed assigned tasks according to deadlines and all procedures were performed according to protocols.
Funding Support	Not applicable
Name	**Dr. Jessica Muniga**
Project Role	Research Veterinarian
Researcher Identifier	Not applicable
Nearest person month worked	6
Contribution to Project	Dr. Muniga performed Hypothesis 1 animal procedures; collected Hypothesis 1 mouse data; assisted with animal protocol amendments, project planning, and procurement of supplies; and set up and performed femoral artery catheterizations with 40 development mice.
Funding Support	Not applicable
Name	**Dr. Zhuowei Li**
Project Role	Research Technician
Researcher Identifier	Not applicable
Nearest person month worked	5
Contribution to Project	Dr. Li assisted with animal protocol amendments, project planning, and procurement of supplies; assisted Dr. Muniga in handling the 40 development mice; and performed plantarflexor muscle function testing.
Funding Support	Not applicable

Name	**Dr. Liyuan (Angi) Zhang**
Project Role	Postdoctoral Scholar
Researcher Identifier	Not applicable
Nearest person month worked	7
Contribution to Project	Dr. Zhang performed Hypothesis 1 animal procedures, collected Hypothesis 1 mouse and tissue data, performed muscle sectioning and antibody-based assays, captured muscle images, and assisted with writing the Hypothesis 1 section of the report.
Funding Support	Not applicable
Name	**Daniela Rincon Cornejo**
Project Role	Project Coordinator
Researcher Identifier	Not applicable
Nearest person month worked	8
Contribution to Project	Ms. Rincon Cornejo managed the grant budget, ordered and tracked laboratory equipment and supplies, assisted with project planning, assisted with laboratory management, and assisted with Hypothesis 1 scheduling.
Funding Support	Not applicable

7.2 Change in the Active Other Support of the PD/PI(s) or Senior/Key Personnel

During Year 01 (20-July-2016 through 19-July-2017), Dr. Schneider's active JWMRP support was 70%.
From 20-July-2016 through 31-December-2016, Dr. Schneider's other active support was 7.5%.
On 01-January-2017, Dr. Schneider's other active support changed from 7.5% to 15%.
On 30-June-2017, Dr. Schneider's other active support changed from 15% to 0%.

7.3 Research Partners

Table 6 lists the organizations that have served as research partners.

Table 6. Research Partners

Organization Name	**University of Cincinnati**
Location of Organization	Cincinnati, Ohio
Partner's contribution to the project	Other – consulted on the hemorrhagic shock and fluid resuscitation

Organization Name	**University of Central Florida**
Location of Organization	Orlando, Florida
Partner's contribution to the project	Collaboration – statistical analysis

Organization Name	**University of Nevada, Las Vegas Howard Hughes College of Engineering**

Location of Organization	Las Vegas, Nevada
Partner's contribution to the project	In-kind support – developed software for leukocyte quantification

8.0 SPECIAL REPORTING REQUIREMENTS

8.1 Collaborative Awards
Not applicable

8.2 Quad Chart

The Effect of Hypobaria on Muscle Inflammation and Regeneration after Injury and Hemorrhagic Shock

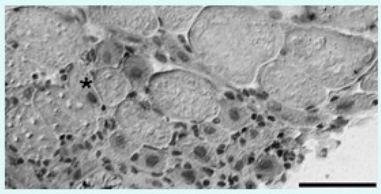
JW150007, Joint Warfighter Medical Research Program
W81XWH-16-1-0150
PI: Barbara St. Pierre Schneider **Org:** University of Nevada, Las Vegas **Award Amount: $5,558,801**

Study/Product Aim(s)

•The overall study aim is to examine the effect of hypobaria on muscle inflammation and regeneration after injury and hemorrhagic shock with fluid resuscitation.

Approach

An experimental, laboratory approach will be used to address the study aim. To examine muscle inflammation and regeneration, four major methods will be used: for proteins, immunohistochemistry flow cytometry; and for messenger RNA levels, polymerase chain reaction. Flow cytometry and serum bioassays will also be used to examine systemic inflammation. Finally, muscle function testing will be performed to examine functional recovery.



Regenerating fibers (*) in crush-injured quadriceps muscle (bar = 100 microns)

Year 01 Accomplishment: Animal approval, personnel hiring, completion of Hypothesis 1 animal procedures and tissue analysis.

Timeline and Cost

Activities	17	18	19	20
Animal Approval & Hiring				
Hypothesis 1 Testing				
Hypothesis 2 Testing				
Hypothesis 3 Testing				
Estimated Budget	**$1,271,577**	**$1,406,671**	**$1,447,661**	**$1,432,892**

Updated: 15-August-2017

Goals/Milestones

CY17 and 18 Goals – Animal approval and personnel hiring, and model refinement
☐ Set up hemorrhagic shock model.
☐ Examine inflammation activation of fluid resuscitation.

CY18 and CY19 Goals – Effect of hypobaria after injury
☐ Investigate effect of hypobaria on crush muscle injury and hemorrhagic shock in 190 subjects.

CY19 and CY20 Goals – Effect of hypobaria after injury
☐ Investigate effect of hypobaria on crush muscle injury and hemorrhagic shock in 338 subjects.

Comments/Challenges/Issues/Concerns
- Delays in animal approval, personnel hiring, and setting up hemorrhagic shock/fluid resuscitation model

Budget Expenditure to Date
Projected Expenditure: $5,558,801
Actual Expenditure: $497,650.14

9.0 APPENDICES
None